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## Structure, function and evolution of the microbiota thriving at the root-soil interface

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# Structure, function and evolution of the microbiota thriving at the root-soil interface

Davide Lab



Davide Bulgarelli PhD,  
College of Life Sciences  
Annual Research Symposium  
Crieff March 20<sup>th</sup>, 2015

# **The menu**

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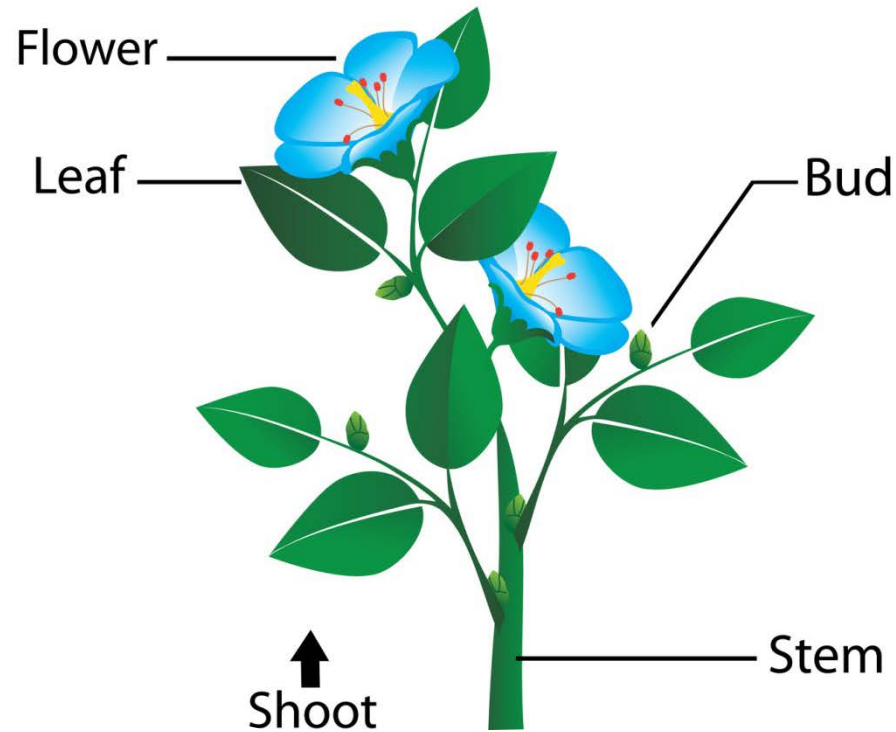
**The root-soil interface**

**The barley microbiota**

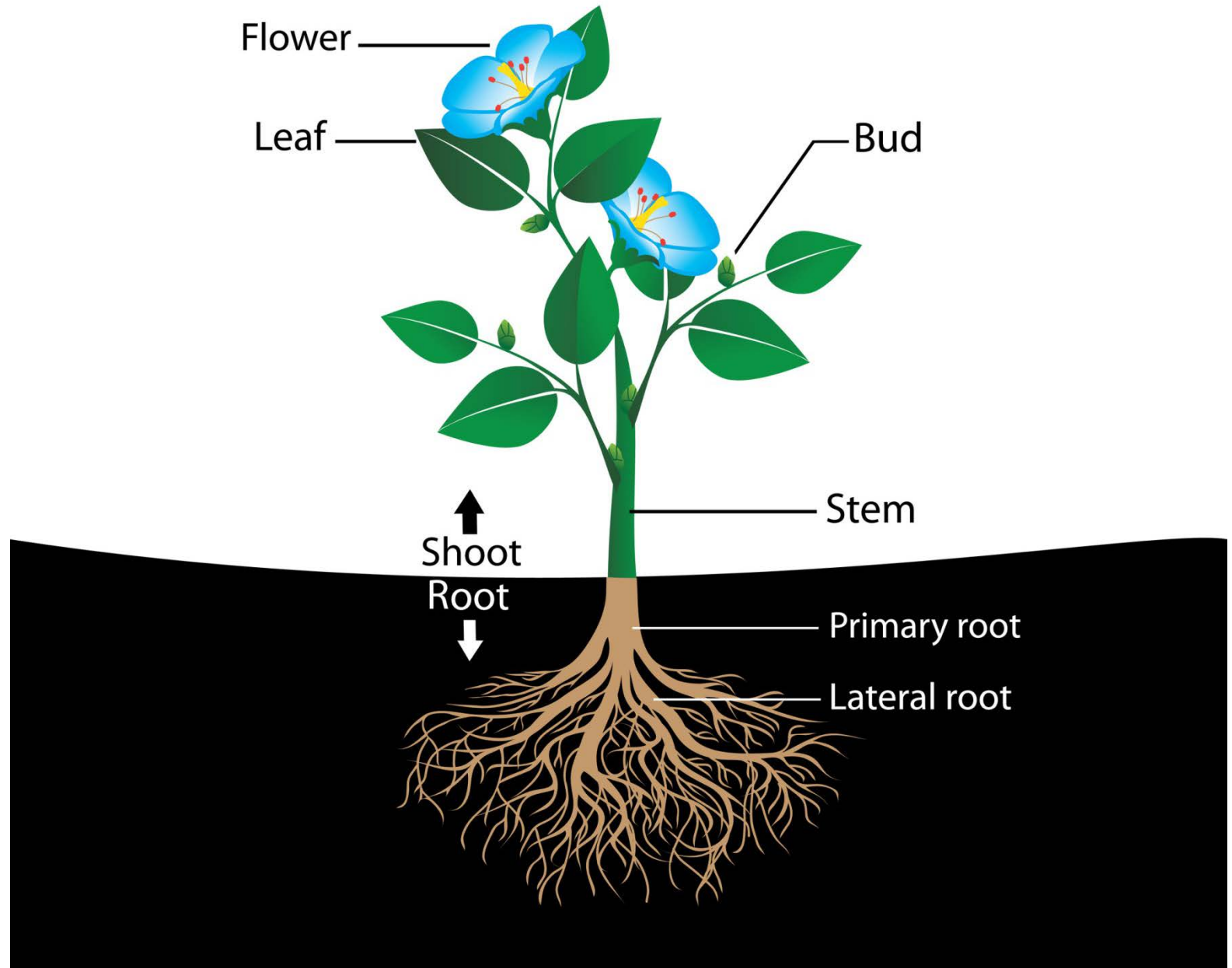
**What's next?**

# Spring is coming...

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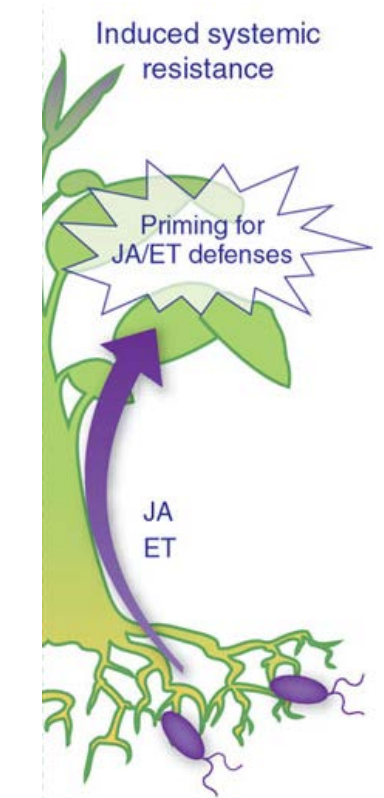
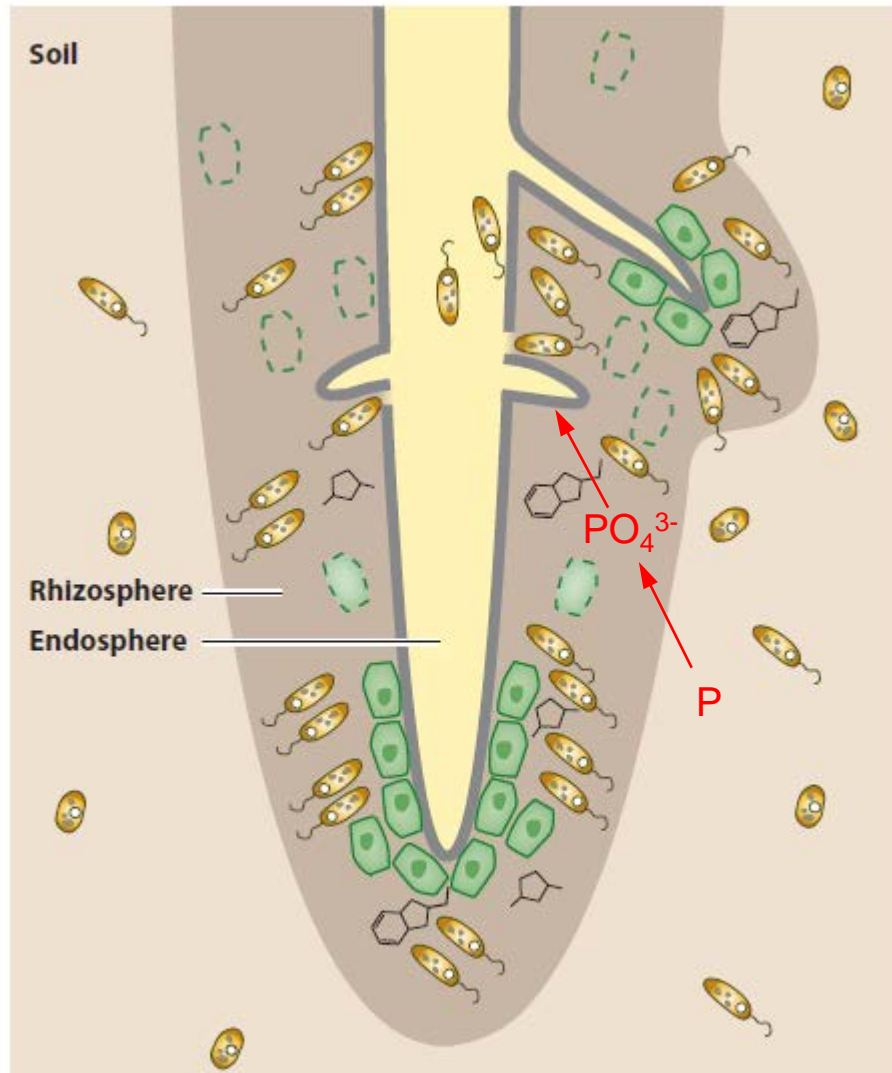


# This is a plant!





# “Plants wear their guts on the outside”



Plant Microbiota

Structure? Functions? Host Control?

# Are plants and toothpicks different?

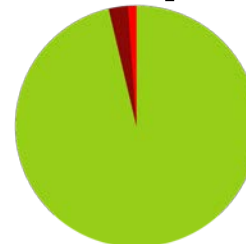
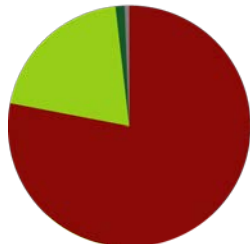


Microbiota

Root

Shared

Toothpick

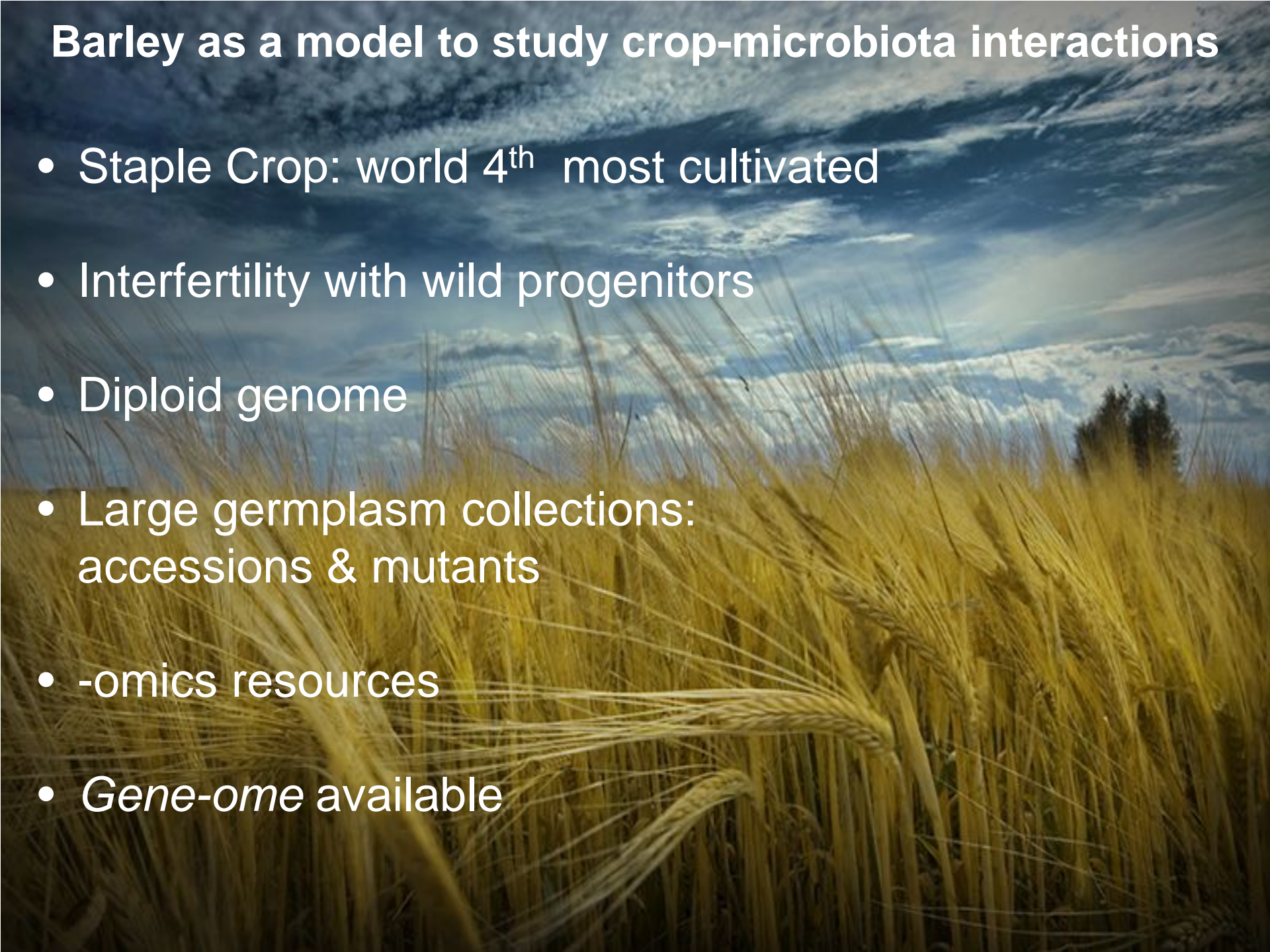


■ Proteobacteria   ■ Actinobacteria   ■ Bacteroidetes   ■ Planctomycetes



# Barley as a model to study crop-microbiota interactions

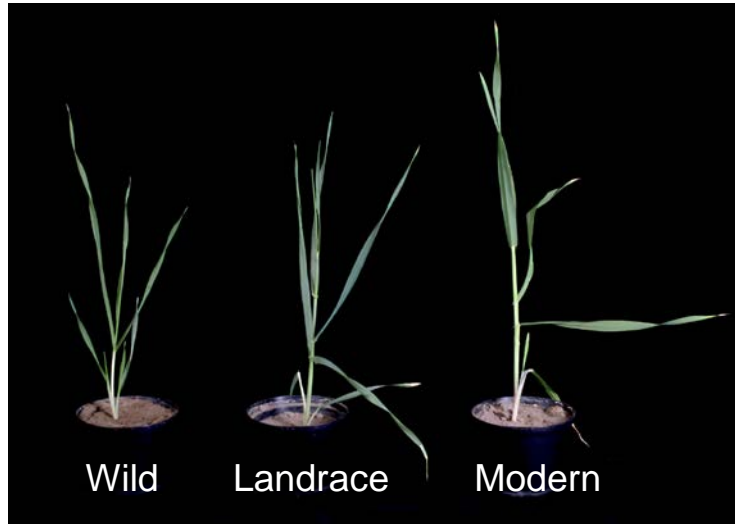
- Staple Crop: world 4<sup>th</sup> most cultivated
- Interfertility with wild progenitors
- Diploid genome
- Large germplasm collections: accessions & mutants
- -omics resources
- *Gene-ome* available





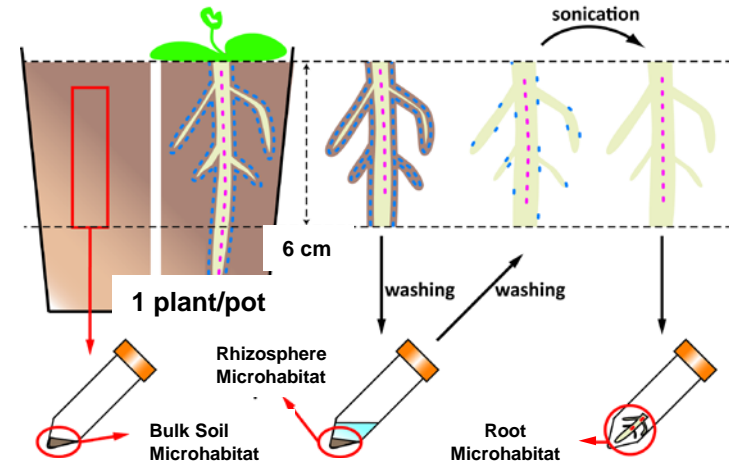
# Do crop plants and their wild relatives recruit different microbiota?

## I) Plants grown in natural soil

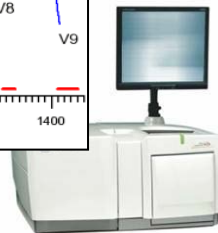
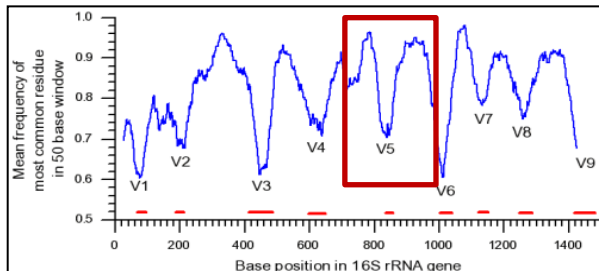


Golm soil, 2 seasonal samplings

## II) Sample fractionation & DNA isolation



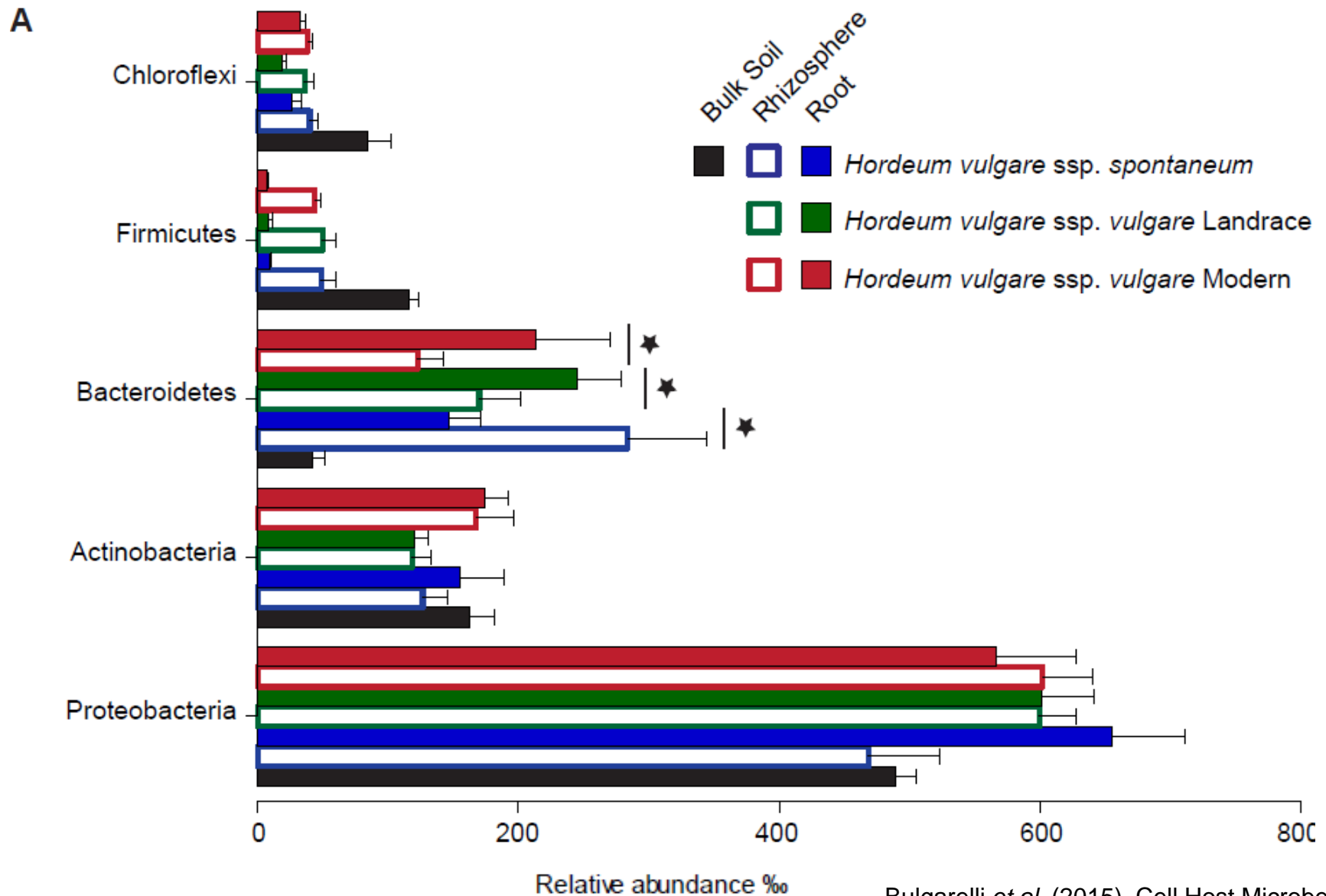
## III) Pyrosequencing of the 16S rRNA gene



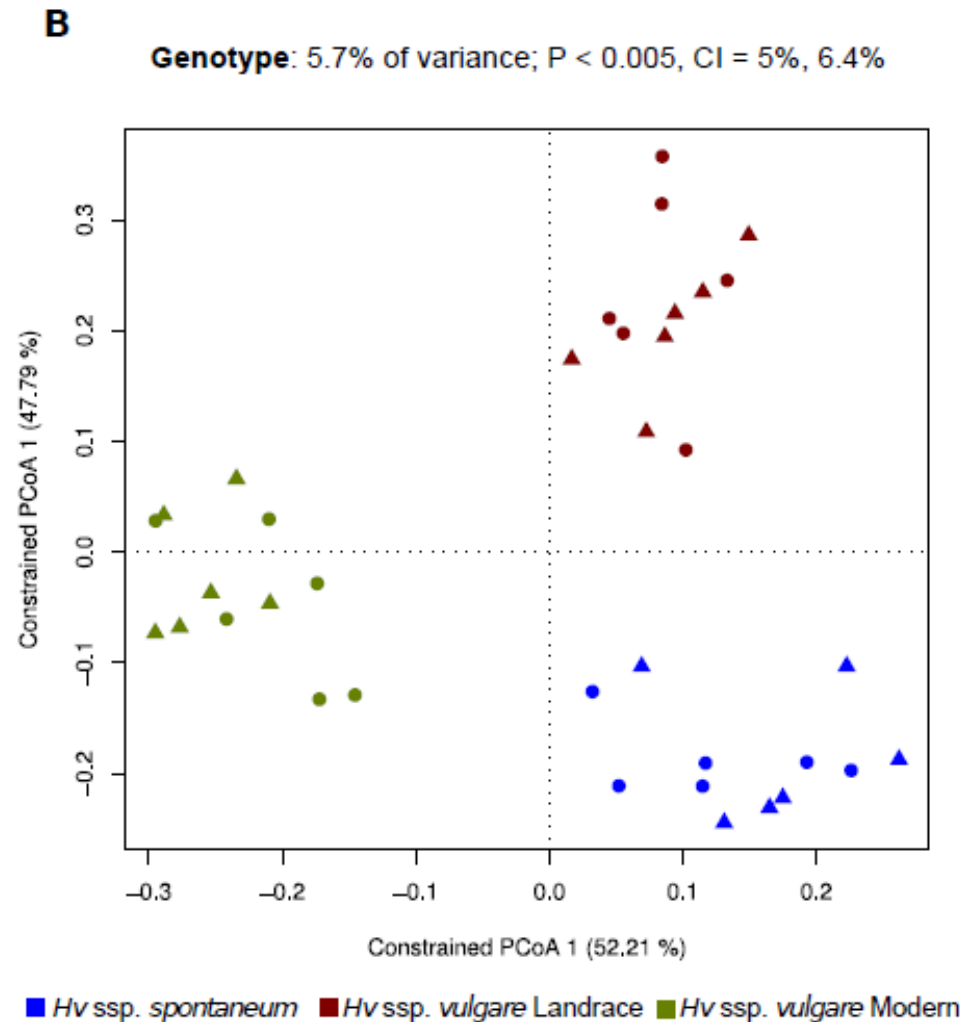
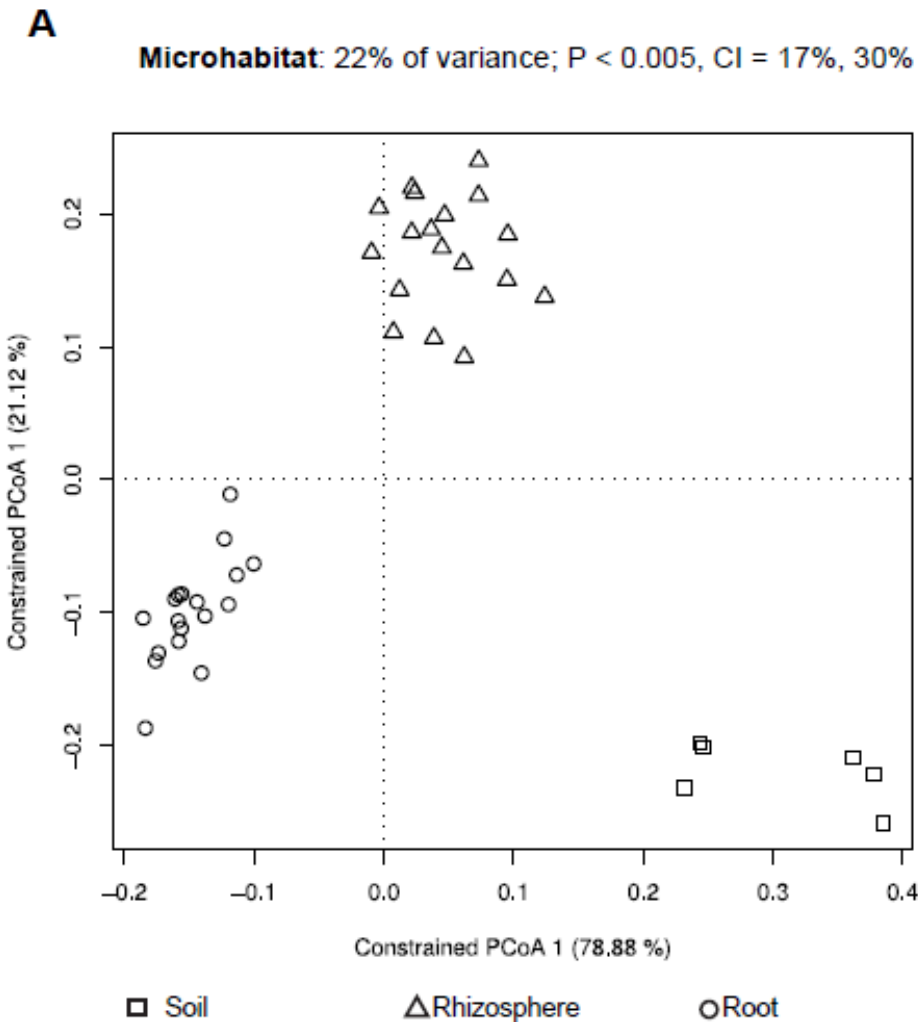
## IV) Sequence analysis

- Operational taxonomic units (**OTUs**) definition
- Taxonomic information
- Structural analysis using ecological indices and multivariate statistics

# The barley bacterial microbiota is a gated community

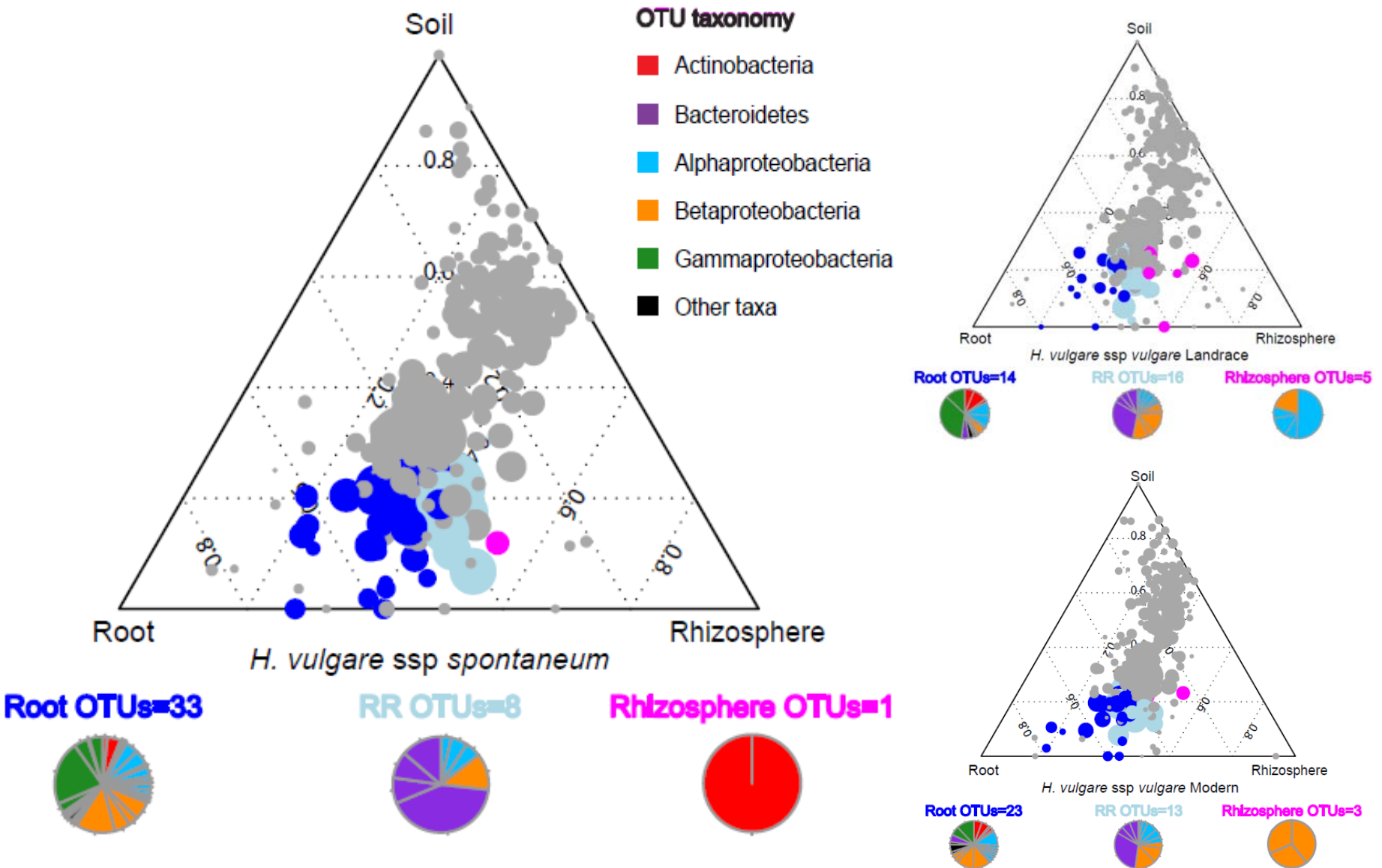


# The habitat determines the structure of the microbiota... ...and the host genotype fine tunes its composition

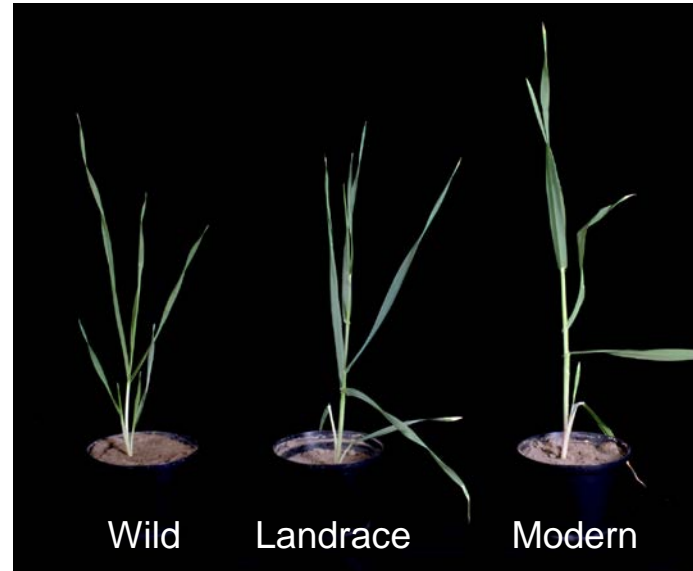




# Barley shapes the root and rhizosphere microbiota

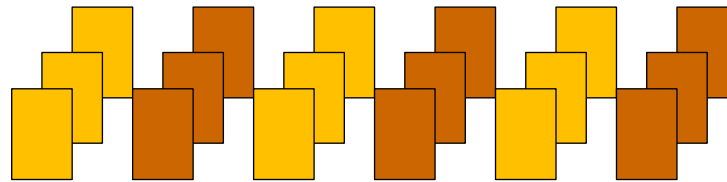


# Shotgun metagenomics of the barley rhizosphere: the barley microbiome



Golm soil #1 

Golm soil #2 



Rhizosphere DNA  
2x soils; 3x reps



Pooling



2x100bp, 25M PE reads x  
genotype X soil

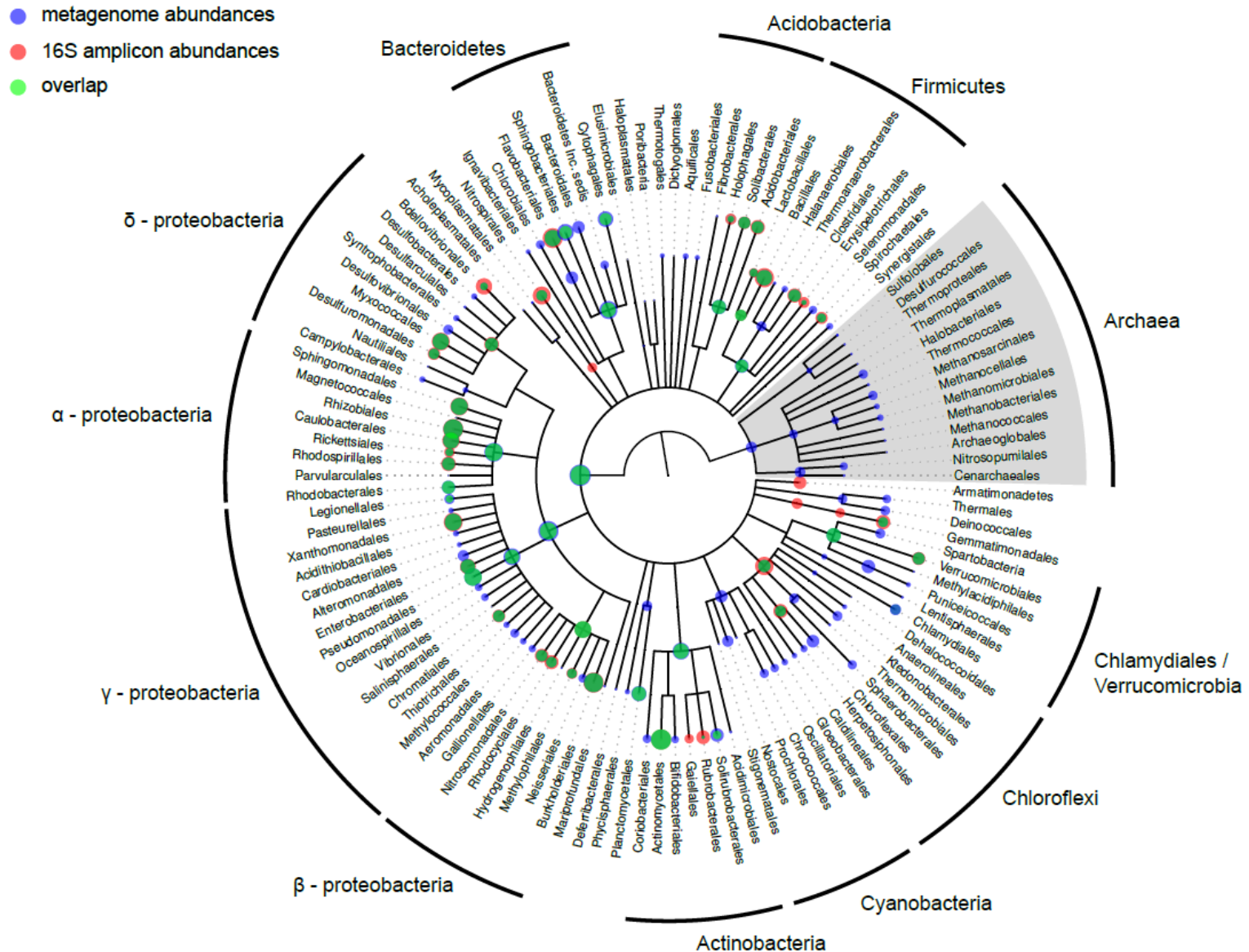


QC, assembly, binning,  
annotation

Microbiota taxa & functions



# Bacteria dominate the annotated barley microbiome





# Amplicon vs. Metagenomics sequencing

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16S rRNA gene abundances  
metagenomics



Pearson  $r = 0.89$   
 $P < 2.15E-14$

16S rRNA gene abundances  
amplicon sequencing



Pearson  $r = 0.86$   
 $P < 1.75E-12$

Taxa abundances  
metagenomics

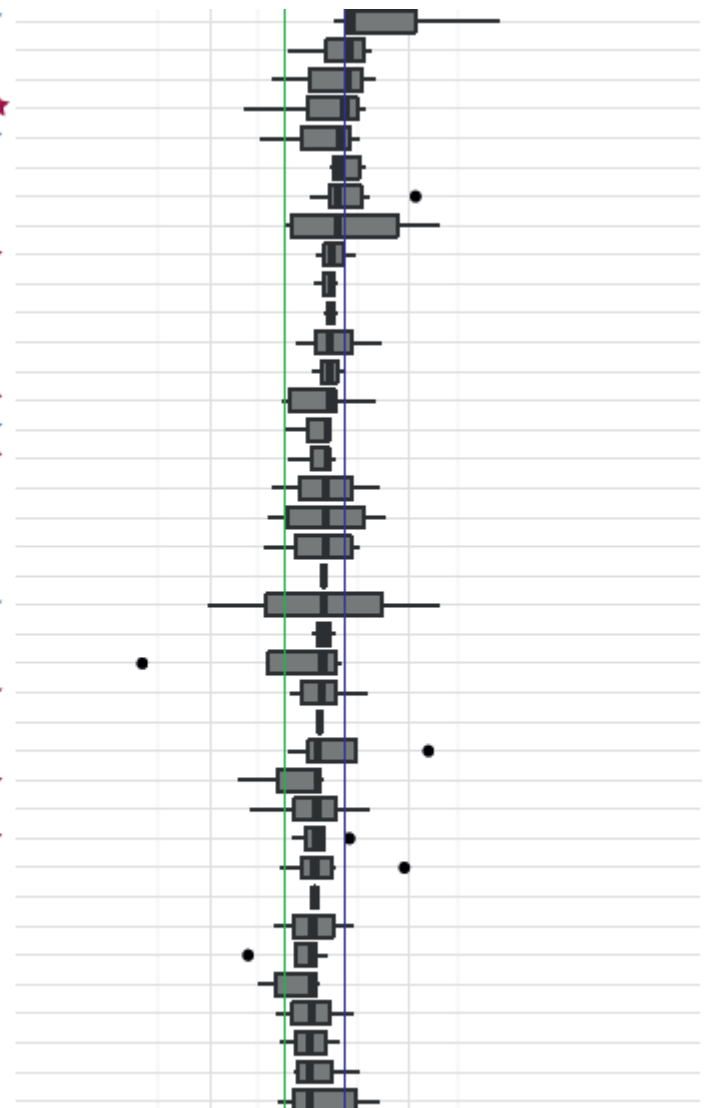
# Linking structure and function

**Table 1. Functions enriched in root and rhizosphere-associated bacterial taxa**

Functional Category	P value <sup>*</sup>
→ Protein secretion system Type III	0.0013
→ Adhesion	0.0014
→ Regulation of virulence	0.0016
→ Siderophores	0.0016
Secretion	0.0053
Transposable elements	0.0138
Periplasmic Stress	0.0152
Sugar Phosphotransferase Systems	0.0209
→ Bacteriophage integration excision lysogeny	0.0300
Invasion and intracellular resistance	0.0300
Detoxification	0.0339
→ Protein secretion system Type VI	0.0339
Acid stress	0.0356

# Positive selection in the barley rhizosphere

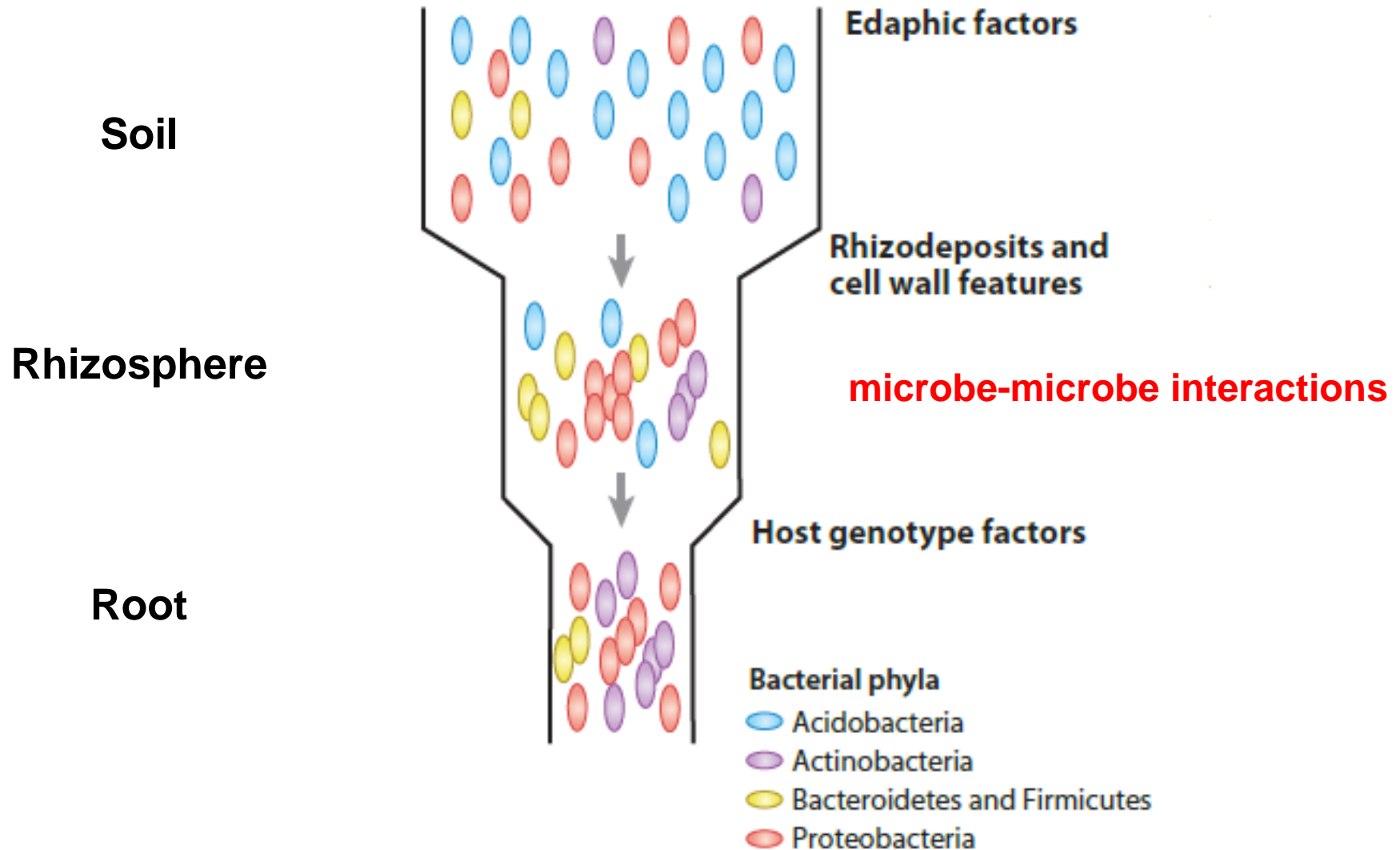
WXG100\_ESAT6: WXG100 family type VII secretion target ★  
 Rv1155\_F420: PPOX class probable F420-dependent enzyme  
 Gneg\_AbrB\_dup: membrane protein AbrB duplication  
 VI\_zyme: type VI secretion system lysozyme-like protein ★  
 tolA\_full: protein TolA ★  
 pre\_pil\_HX9DG: prepilin-type processing-associated H-X9-DG domain  
 AmmeMemoSam\_B: AmmeMemoRadiSam system protein B  
 Fsuc\_second: TIGR02147 family protein  
 phageSPP1\_gp7: phage head morphogenesis protein, SPP1 gp7 family ★  
 Rv2061\_F420: PPOX class probable F420-dependent enzyme, Rv2061 family  
 TIGR02231: conserved hypothetical protein  
 TIGR03083: uncharacterized Actinobacterial protein  
 Dyp\_perox\_fam: Dyp-type peroxidase family  
 phge\_HK97\_gp10: phage protein, HK97 gp10 family ★  
 flagell\_FliJ: flagellar export protein FliJ ★  
 psiM2\_ORF9: phage uncharacterized protein, C-terminal domain ★  
 M6dom\_TIGR03296: M6 family metalloprotease domain  
 myb\_SHAQKYF: myb-like DNA-binding domain, SHAQKYF class  
 MYXO-CTERM: MYXO-CTERM domain  
 pepcterm\_hypo\_1: uncharacterized protein, PEP-CTERM system associated  
 type\_III\_tyeA: type III secretion effector delivery regulator, TyeA family ★  
 phosphate/phosphite/phosphonate ABC transporters, periplasmic binding protein  
 TIGR00242: protein MraZ  
 YgiT\_finger: YgiT-type zinc finger domain ★  
 ICE\_PFL4669: integrating conjugative element protein  
 Mxa\_TIGR02265: Myxococcales-restricted protein  
 phage\_pRha: phage regulatory protein ★  
 diphth2\_R: diphthamide biosynthesis enzyme Dph1/Dph2 domain  
 TIGR02241: conserved hypothetical phage tail region protein ★  
 RrgB\_K2N\_iso\_D2: fimbrial isopeptide formation D2 domain  
 antibiot\_sagB: SagB-type dehydrogenase domain  
 yiiD\_Cterm: putative thioesterase domain  
 2\_5\_ligase: 2'-5' RNA ligase  
 pilus\_cpaD: pilus (Caulobacter type) biogenesis lipoprotein CpaD  
 plancto\_dom\_1: Planctomycetes uncharacterized domain  
 arsC\_related: transcriptional regulator, Spx/MgsR family  
 PGA\_PgaD: poly-beta-1,6-N-acetyl-D-glucosamine biosynthesis protein PgaD  
 TRAP\_TAXI: TRAP transporter solute receptor, TAXI family



An evolutionary arms race in the barley rhizosphere?



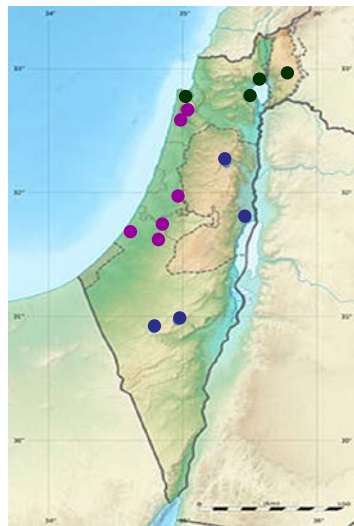
# A revised two-step process for the microbiome differentiation:



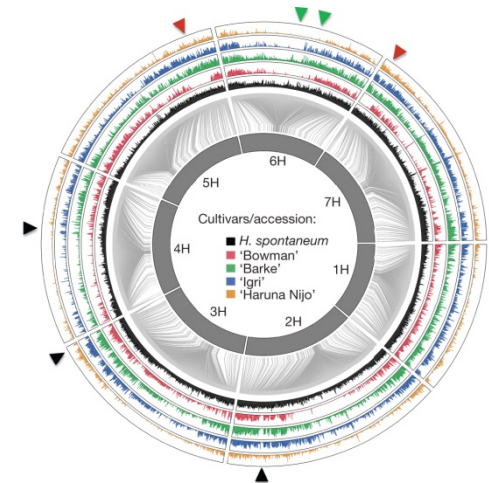
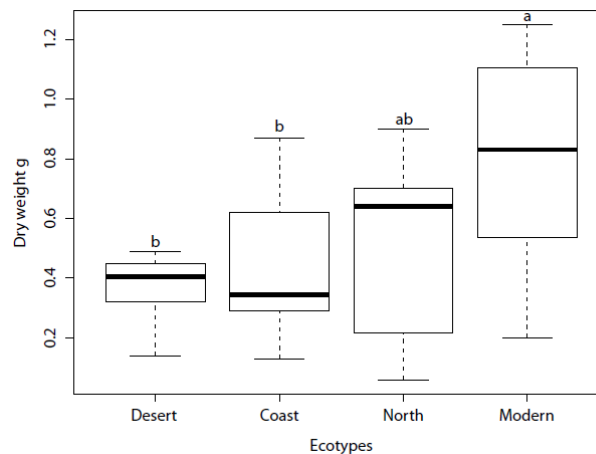
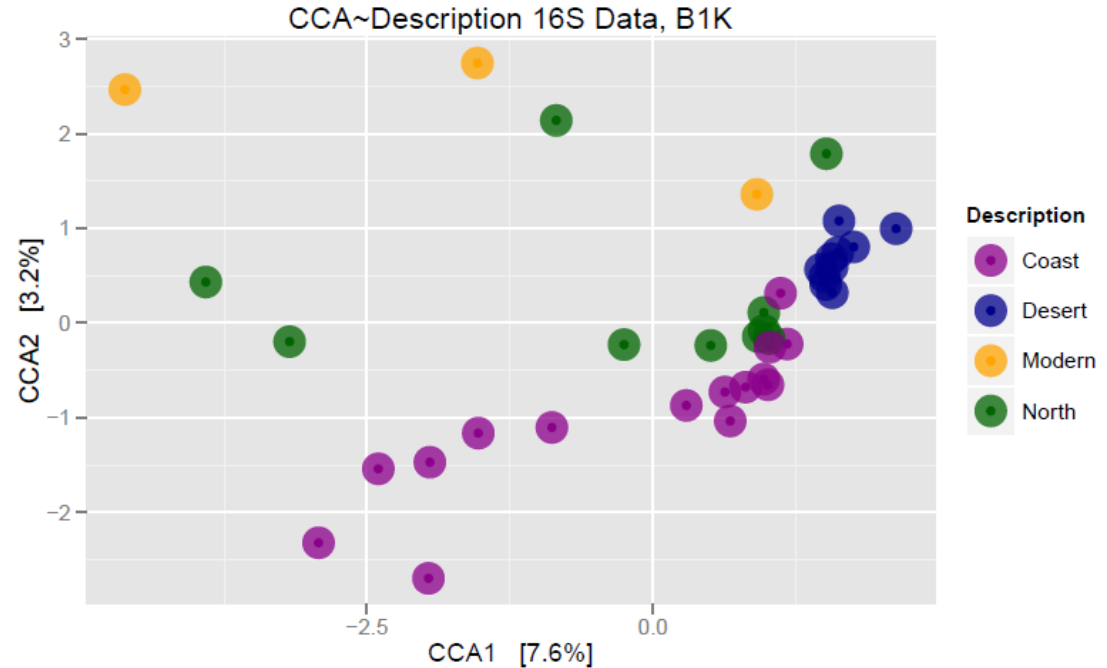
# An on going co-evolutionary experiment



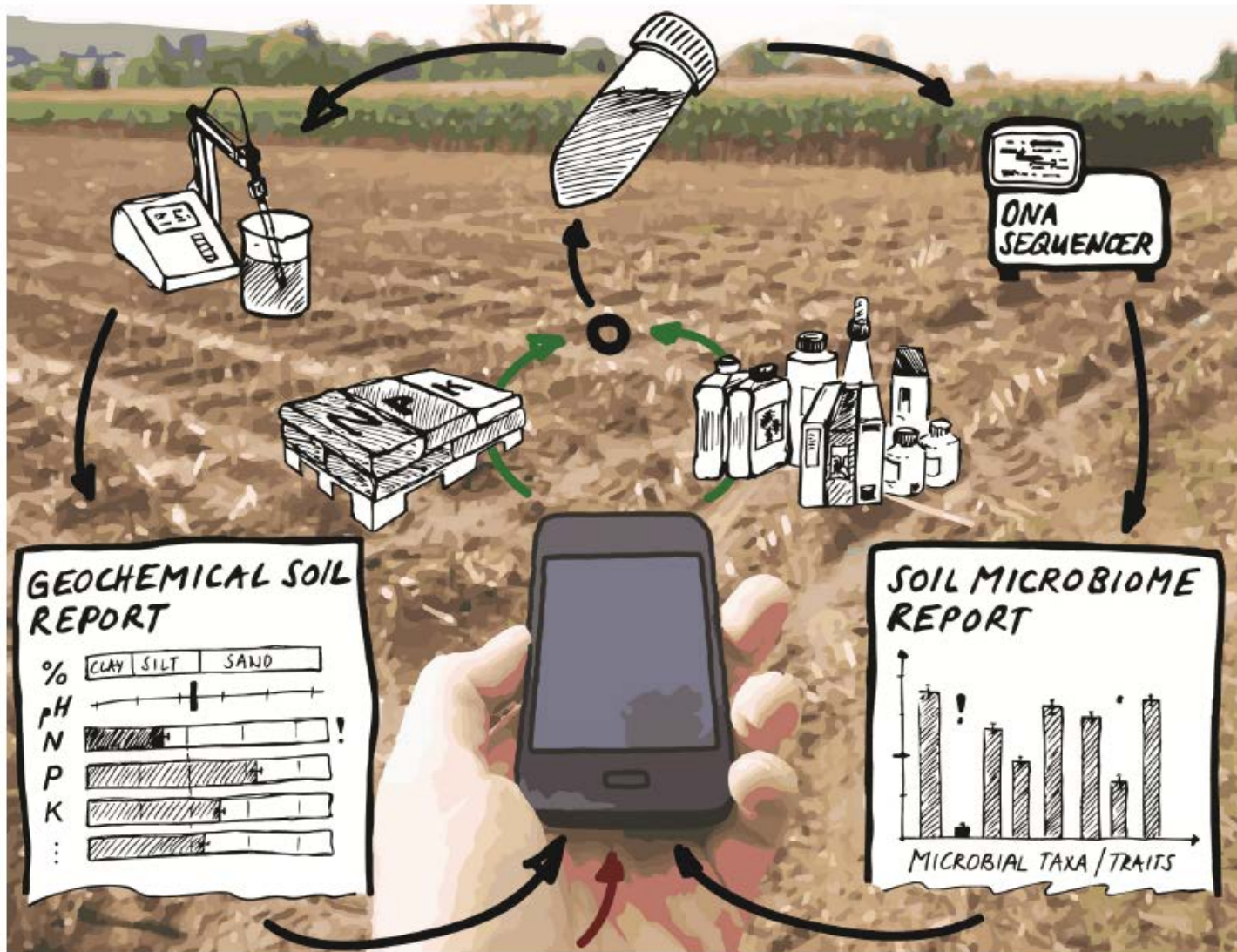
Wild barley ecotypes



- North
- Coast
- Desert



# Next-generation agriculture





# Credits

## **Davide Lab**

Katharin Balbirnie

Rodrigo Alegria Terrazas

Looking for a PhD? Your name can be here...

## **Paul Schulze-Lefert (MPI Cologne)**

Ruben Garrido Oter

Anne Vortkamp

Isa Will

Maren Winnacker

## **Alice McHardy (HZI Braunschweig)**

Philipp Münch

Johannes Dröge

Aaron Weimann

Yao Pan

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Eric Paterson (JHI Aberdeen)

Elizabeth Baggs (U Aberdeen)

Eyal Fridman (HU Jerusalem)

## ***Funding:***



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